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EFFECT OF SURFACE CHEMICALS ON INTRAGUILD PREDATION OF EGGS OF TWO APHIDOPHAGOUS LADYBEETLES, *Coccinella undecimpunctata* L., AND *Cydonia visina ISIS* CR. (COLEOPTERA: COCCINELLIDAE)

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ABSTRACT

Intraguild predation (IGP) on egg and the role of surface chemicals in two aphidophagous coccinellids species, *C. undecimpunctata* and *C. visina isis* were examined. The first instar larvae of both species prefer to eat washed heterospecific eggs than unwashed eggs. Surface chemicals appear to play a major role for the preference of washed eggs, as the ladybeetle behavior was reversed when these chemicals were remained. The surface chemicals present on the eggs appears to reduce intraguild predation. The green lacewing, *Chrysoperla carnea* (Stephens) in its first instar consumed *C. undecimpunctata* and *C. visina isis* eggs without showing any preference for either washed or unwashed treatments, although we observed that the washed eggs were eaten more than those unwashed. The average number of coccinellid eggs consumed by the first instar larvae of other predator species was determined and the results are discussed.

INTRODUCTION

The first instar larvae of aphidophagous coccinellids (Coleoptera: Coccinellidae) are under strong selection pressure to find food soon after hatching. They can readily eat unhatched or late hatched eggs of their own batches due to the presence of trophic eggs and asynchronous egg hatching (Osawa, 1992). The eggs of certain aphidophagous ladybirds, viz. *Coccinella septempunctata* L. may be protected against non-sibling cannibalism and heterospecific predation (Hemptinne *et al*., 2000b). However, eggs of certain species can readily be victimized by hungry ladybeetles.

Eggs are generally assumed to be highly nutritious with yolk being a rich protein source for developing embryonic larvae. They are, therefore, a high quality food source meeting the nutritional requirements of the neonates better than alternative prey (Gagne *et al*., 2002).

The same egg can be nutritive to one ladybird species while toxic to the other (Hemptinne *et al*., 2001; Burgio *et al*., 2002; Santi *et al*., 2003). If neonates possess the ability to discriminate amongst their prey (Gagne *et al*., 2002), it is expected that they would selectively consume the more suitable food: unrelated conspecific or heterospecific eggs. Keeping in view the nutritional benefits of conspecific eggs (Snyder *et al*., 2000).

Chemicals present on the surface of eggs may also influence the cannibalism and predation by later larval instars and adults (Agarwala and Dixon, 1993; Hemptinne *et al*., 2000b, 2001). They play a major role in attraction/repulsion of predators and have relative costs and benefits on predator's fitness (Hemptinne *et al*., 2001). The removal of these chemicals may negate the ability to discriminate amongst conspecific and heterospecific eggs result in random predation of eggs. Clustering of eggs is common in aphidophagous ladybirds (Agarwala and Dixon, 1993). Egg clustering might increase an individual's chance of avoiding predatory attacks (Kiltie, 1980). Clustered eggs may have aposematic properties, deterring intraguild predation (IGP) (Agarwala and Dixon, 1993) and non-sibling cannibalism (Agarwala *et al*., 1997).

Eggs and younger larvae are more vulnerable to cannibalism by older larvae than vice versa (Agarwala and Dixon, 1992). Similarly, in intraguild predation, a small species is more likely to be the intraguild prey of a large species (Sengonca and Frings, 1985; Lucas *et al*., 1998; Phoofolo and Obrycki, 1998; Hindayana *et al*., 2001). However, in species of ladybird that have overlapping habitat preferences, small species, such as *Adalia bipunctata* L. are more toxic to large species, such as *C. septempunctata*, and this reduces the incidence of the small species being eaten by the large species (Agarwala and Dixon, 1992; Agarwala *et al*., 1998; Hemptinne *et al*., 2000a). Thus, vulnerable species may be protected chemically from intraguild predation.

Within the community, predation risk of individuals may dependent upon factors such as their mobility and the semiochemicals (allomones) that they produce as demonstrated in Coccinellidae (Felix and Soares, 2004; Omkar *et al*., 2004). However, Hemptinne *et al*. (2000b) found that some alkaloids are present in *A. bipunctata* and *C. septempunctata* eggs, which may contribute to reducing intraguild predation as in other ladybird species (Omkar *et al*., 2004).

All stages of ladybeetle contain similar concentrations of alkaloids (Pasteels *et al*., 1973) that are thought to be responsible for their toxicity. Eggs and hatching larvae are relatively easy to obtain compared to other developmental stages. Therefore, in this study, IGP by hatching ladybeetle and Chrysopid larvae fed eggs of other species were determined to examine interspecific egg predation among predator species and the egg toxicity effect of two ladybeetles, *C. undecimpunctata* and *C. visina isis* on the incidence of predation between these species.

MATERIALS AND METHODS

To obtain eggs, pairs of adults were kept in plastic Petri dishes (9 cm in diameter), each containing a piece of filter paper, and fed daily with aphids, *Aphis craccivora* Koch. Any eggs laid on the filter paper were removed and placed in other Petri dishes (9 cm in diameter).

To evaluate the effect of surface alkanes on IGP in different predators, two clusters of C. *undecimpunctata* eggs were individually in Petri dishes (9 cm in diameter), the first cluster contained freshly laid eggs and the second were washed in n-hexane for two minutes to remove the surface alkanes and dried using a filter paper. Hexane was chosen as a solvent because a preliminary study indicated that it does not penetrate eggs during two minutes extraction period, but only removes chemicals present on the egg surface (Chan, 1995).

A six hours starved first instar larvae of *C. visina isis* and *C. carnae* were introduced individually into Petri dishes which containing treated (surface washed eggs) and untreated (freshly laid eggs) clusters of 10 *C. undecimpunctata* eggs. Each treatment (washed or unwashed) was replicated 10 times. The experiment was conducted under laboratory conditions at 28.0 ± 2.0°C, 75.0 ± 5 % RH and photoperiod of 14L: 10D. The first instar larva of the two species was allowed to feed separately on eggs and observations on egg IGP were taken after 24 h. After 24 h, the larvae were removed and the number of egg consumed was determined by counting the number of remaining and left over eggs.

In the same way, the experiment was repeated using eggs of *C. visina isis*, both treated (surface washed eggs) and untreated (unwashed eggs), and with the first instar larvae of *C. undecimpunctata* and *C. carnae* as IG predators.

Data analysis:

The data of the average number of washed or unwashed eggs consumed were subjected for one way analysis of variance (ANOVA), and the means were separated using Duncan's Multiple Range Test (Costat Software, 2004).

RESULTS AND DISCUSSION

To examine chemical protection of ladybeetle eggs, the reluctance of larvae to eat the eggs of other species of ladybeetle was determined. The average number of coccinellid eggs consumed by the first instar larvae of other species is given in (Figures 1 and 2).

After 24 hours, the consumption of *C. undecimpunctata* eggs by the first instar larvae of *C. vivina isis* varied significantly between the two washed and unwashed treatments (Table 1). Average numbers of washed and unwashed eggs fed to larvae were 7.2 ± 0.49 (72%) and 3.1 ± 0.23 (31%), respectively (Figure 1). There was a highly significant difference of *C. vivina isis* washed eggs (7.9±0.59) were consumed compared with unwashed eggs consumed by *C. undecimpunctata* (4.2±0.25) (Table 2, Figure 2). The percentage of *C. vivina isis* unwashed eggs consumed by larvae of *C. undecimpunctata* (42%) was significantly lower than percentage of washed consumed eggs (79%) (Figure 2). In the two species of ladybeetles, larvae preferentially attacked and ate the clusters containing washed heterospecific eggs. The first instar larvae of *C. undecimpunctata* and *C. vivina isis* consumed heterospecific eggs without surface chemicals more than heterospecific eggs containing surface chemicals. Thus, chemicals present on the egg surface deter larvae from engaging in IGP. Agarwala and Dixon

(1992) reported reluctance by the coccinellids, *A. bipunctata* and *C. septempunctata* to eat eggs of the other species.

Table 1. One way analysis of variance (ANOVA) for the effects of IGP on eggs of *C. undecimpunctata* washed or unwashed in n- hexan by the first instar larvae of *C. vivina isis* after 24h under laboratory condition.

Source	SS	df	MS	F	P
Mean effects	84.05	1	84.05	57.09	0.000***
Error	26.5	18	1.472	-	-
Total	110.55	19	-	-	-

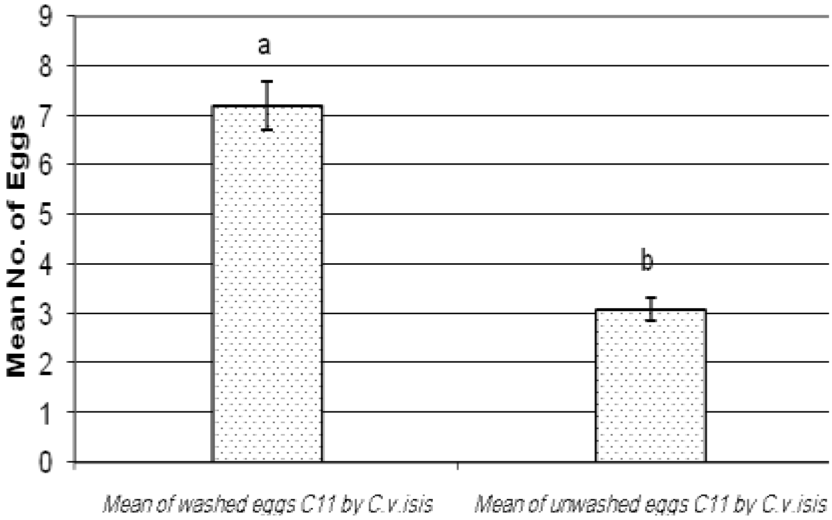

Figure 1. Mean numbers ± SEM of washed or unwashed *C. undecimpunctata* eggs consumed by the first instar larvae of *C. vivina isis* after 24h under laboratory conditions. Means followed by the same small letter are not significantly different at the 1% level of probability (Duncan's Multiple Range Test).

Table 2. One way analysis of variance (ANOVA) for the effects of IGP on eggs of *C. vivina isis* washed or unwashed in n- hexan by the first instar larvae of *C. undecimpunctata* after 24h under laboratory condition.

Source	SS	df	MS	F	P
Mean effects	68.45	1	68.45	33.76	0.000***
Error	36.5	18	2.028	-	-
Total	104.95	19	-	-	-

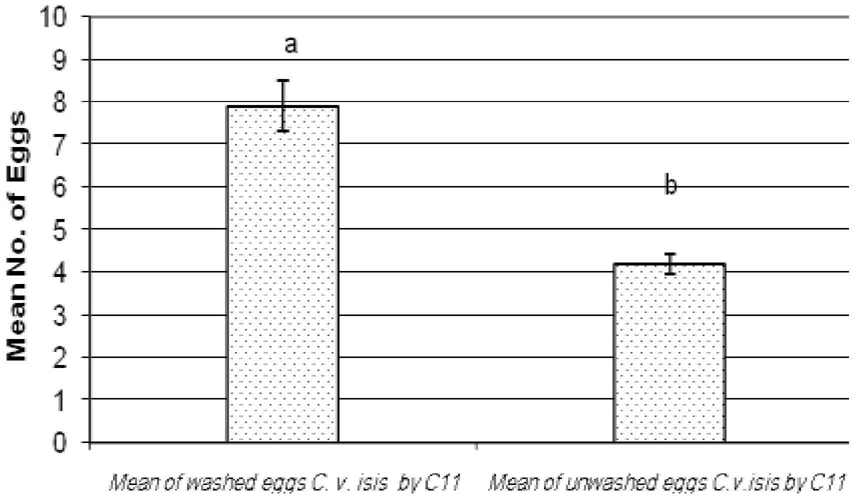

Figure 2. Mean numbers ± SEM of washed or unwashed *C. vivina isis* eggs consumed by the first instar larvae of *C.undecimpunctata* after 24h under laboratory conditions. Means followed by the same small letter are not significantly different at the 1% level of probability (Duncan's Multiple Range Test).

In ladybeetles, it seems that surface chemistry of heterospecific eggs might be less effective between species. Insect eggs are rich in cholesterols (MacDonald *et al*., 1990) and it appears that the egg cholesterols are species-specific. Surface chemistry of different insects varies (Carlson *et al*., 1999; Nielsen *et al*., 1999) and is thus likely to be more similar within than between species. Hemptinne *et al*. (2000a) suggest it is highly unlikely that coccinellid larvae would encounter sufficient numbers of coccinellid eggs (e.g., for predation and/or cannibalism) in the field to complete development from first instar to adult.

After 24 hours, the first instar larvae of *C. carnae* attacked a higher percentage of all washed and unwashed eggs of *C.undecimpunctata* {8.1 ± 0.57 (81%) and 6.9 ± 0.23 (69%), respectively} (Table 3, Figure 3) compared with the percentage of eggs attacked by ladybeetles larvae.

Table 3. One way analysis of variance (ANOVA) for the effects of IGP on eggs of *C. undecimpunctata* washed or unwashed in n- hexan by the first instar larvae of *C. carnae* after 24h under laboratory condition.

Source	SS	df	MS	F	P
Mean effects	7.2	1	7.2	3.83	0.0569 ns
Error	33.8	18	1.88	-	-
Total	41	19	-	-	-

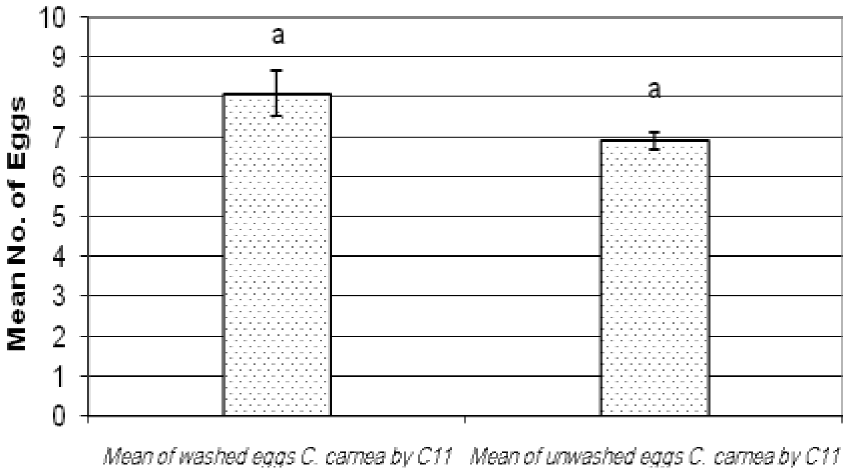

Figure 3. Mean numbers ± SEM of washed or unwashed *C. undecimpunctata* eggs consumed by the first instar larvae of *C. carnae* after 24h under laboratory conditions. Means followed by the same small letter are not significantly different at the 1% level of probability (Duncan's Multiple Range Test).

There were 8.4 ± 0.45 (84%) and 7.5 ± 0.17 (75%) of *C. vivina isis* washed and unwashed eggs consumed by the first instar larvae of *C. carnae* respectively, after 24 hours (Table 4, Figure 4). The percentage of egg consumed by larvae of *C. carnae* was high but not significantly different between the two washed and unwashed treatments. Thus, *C. carnae* is likely to have a negative impact upon this coccinellid species through intraguild egg predation, especially when aphid populations decline.

The toxicity of eggs not only varies among ladybeetle species but also the impact of that toxicity on the intraguild predator varies across species. One hypothesis that this variation reflects the degree and intensity with which any two species may interact through intraguild predation (Sato and Dixon, 2004).

The mouthparts of *C. carnea* larvae and *Orius laevigatus* (Fieber) nymphs and adults allow these species to perforate the egg chorion, and then suck the contents from the egg. If the chorion of a ladybird egg was broken by the mouthparts of the lacewing larva, this stopped the development of the embryo (Santi and Maini, 2006). Intraguild predation has the potential to be a major source of egg mortality among ladybirds, and chemical defense of eggs appears important often in reducing the incidence of such intraguild predation (Agarwala and Dixon, 1992 & 1993; Yasuda and Shinya, 1997; Schellhorn and Andow, 1999; Hemptinne *et al*. 2000 a & b; Agarwala and Yasuda, 2001; Sato and Dixon, 2004; Cottrell, 2004 & 2005).

Table 4. One way analysis of variance (ANOVA) for the effects of IGP on eggs of *C. vivina isis* washed or unwashed in n- hexan by the first instar larvae of *C. carnae* after 24h under laboratory condition.

Source	SS	df	MS	F	P
Mean effects	4.05	1	4.05	3.49	0.0782 ns
Error	20.9	18	1.16	-	-
Total	24.95	19	-	-	-

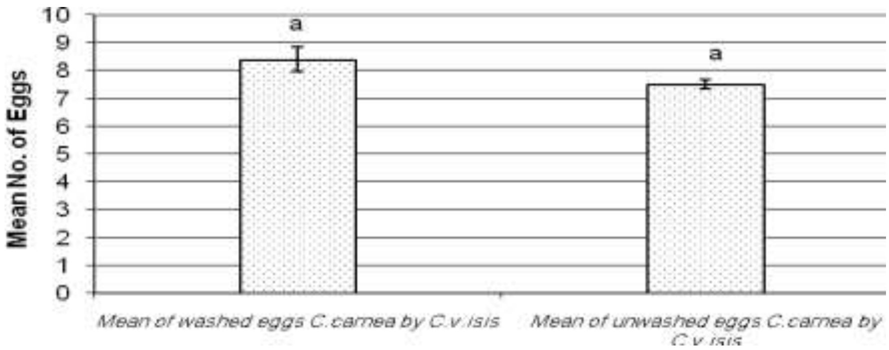

Figure 4. Mean numbers ± SEM of washed or unwashed *C. vivina isis* eggs consumed by the first instar larvae of *C. carnae* after 24h under laboratory conditions. Means followed by the same small letter are not significantly different at the 1% level of probability (Duncan's Multiple Range Test).

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تأثير وجود الكيماويات الموجودة على سطح بيض نوعين من مفترسات أبو العيد (أبوالعيد ١١ نقطة وأبوالعيد الأسود) على إفتراسه من قبل المفترسات الأخرى للمَّن تحت الظروف المعملية

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تم دراسة إفتراس بيض كل من أبو العيد ١١ نقطة و أبو العيد الإسود من قبل المفترسات الأخرى للمَّن وتأثير تواجد الكيماويات المحيطة بالبيض على هذا الإفتراس وذلك بعد مقارنة نسب إفتراس البيض عند وجود الألكانات الطبيعية المحيطة ببيض أنواع أبو العيد مع نسب إفتراس البيض بعد إستخدام الهكسان كمذيب عضوى لتلك الألكانات المحيطة بالبيض تحت الظروف المعملية.

وقد أوضحت النتائج أن نسب إفتراس يرقات العمر الأول حديثة الفقس لكلا نوعين أبو العيد على بيض النوع الآخر لأبو العيد المعامل بالهكسان كانت أعلى من نسب إفتراس اليرقات للبيض الغير معامل بالهكسان. حيث لعبت الألكانات المحيطة ببيض أبوالعيد دور فى تقليل معدلات الإفتراس بين أنواع أبو العيد المختلفة لها.

بينما إفترست اليرقات حديثة الفقس من أسد المَّن فى عمرها الأول بيض كل من أبو العيد ١١ نقطة و أبو العيد الإسود دون إظهار أى تفضيل بين البيض المعامل والغير معامل بالهكسان، على الرغم من زيادة عدد البيض المعامل بالهكسان الذى تم إفتراسه بواسطة يرقات العمر الأول من أسد المَّن الأخضر عن البيض الغير معامل بالهكسان إلا أن النتائج قد أوضحت عدم وجود فروق معنوية فى الإفتراس بين البيض المعامل والغير معامل بالهكسان.

قام بتحكيم البحث

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